New developments in investigational HDAC inhibitors for the potential multimodal treatment of cachexia

INVESTIGATIONAL HDAC INHIBITORS FOR THE MULTIMODAL TREATMENT OF CACHEXIA

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Abstract

Introduction: Cachexia is a frequent feature of chronic diseases. This syndrome includes loss of body weight, depletion of skeletal muscle mass and altered metabolic homeostasis. Acceleration of protein and energy metabolism, impaired myogenesis and systemic inflammation contribute to cachexia. Its occurrence impinges on treatment tolerance as well as on patient quality of life, however, no effective therapy is still available.

Areas covered in this review: This review will focus on the use of histone deacetylase inhibitors as pharmacological tools to prevent/delay cachexia, with particular reference to muscle wasting.

Expert opinion: Besides their interference with histone acetylation, novel histone deacetylase inhibitors could be considered as exercise mimetics, supporting their use to treat muscle wasting-associated diseases such as cachexia. In addition, the ability displayed by some of these inhibitors in modulating the release of extracellular vesicles from tumor cells might be an interesting tool to interfere with the development of cancer-induced muscle protein depletion. At present there are few clinical trials testing histone deacetylase inhibitors to treat cachexia, reflecting the lack of robust experimental evidence of effectiveness. Further investigation uncovering the pathogenic mechanisms of muscle wasting coupled with the identification of suitable histone deacetylase inhibitors targeting such alterations is needed.

Keywords: cachexia, skeletal muscle wasting, myogenesis, protein breakdown, energy metabolism acetylation/deacetylation

Article highlights

- Despite several potential therapeutic approaches for cachexia have been proposed on the basis of experimental studies, very few have been explored in clinical trials, rendering cachexia a still untreatable condition;
- the balance between protein acetylation and deacetylation, mainly dependent on HAT/HDAC equilibrium, appears crucial in the regulation of muscle homeostasis in both physiology and pathology;
- first generation HDAC inhibitors, characterized by broad action, can be ineffective to prevent/delay cachexia, or should be used at doses that can even become detrimental to the organism;
- several studies suggest that HDAC inhibitors, being able to target specific mechanisms among which myogenesis and to behave as exercise mimetic agents, could be considered pharmacological tools suitable for preventing or at least delaying muscle wasting in chronic diseases;
- novel HDAC inhibitors, more specific or endowed with additional activity could reveal useful to address at least some of the metabolic alterations occurring in cachexia and, in theory, might be included in combination treatment schedules. However, this hypothesis remains to be tested, since the number of clinical trials is still very low;
- since the pathogenesis of cancer cachexia is multifactorial and no effective therapeutic strategies are actually available, a selectively tailored multidirectional approach to cachexia could benefit from the introduction of HDAC inhibitors.

1. Introduction

Several chronic diseases are characterized by the occurrence of cachexia, a multi-organ syndrome that includes, among other features, loss of muscle and adipose tissue mass. Different factors contribute to the onset and progression of cachexia, such as reduced food intake, metabolic alterations and inflammation. The relative relevance of each factor may vary significantly, depending on the underlying chronic disease.

Perturbations occurring in the skeletal muscle appear particularly important in cachectic patients. Indeed, quality of life and tolerance to treatments are markedly impaired when muscle mass and function cannot be maintained at physiological levels, not to talk about the fact that muscle mass reduction below a certain threshold is not compatible with survival. Being the skeletal muscle the main body protein reservoir, changes in muscle size mainly reflect an altered regulation of protein turnover rates. In particular, muscle hypotrophy/atrophy can result from enhanced protein breakdown rates, reduced protein synthesis rates, or both [1]. The vast majority of the studies reported in the literature agree in considering the acceleration of protein degradation as the most relevant feature underlying muscle wasting in cachexia. Such catabolic drive depends on the activation above normal levels of three main intracellular proteolytic systems, namely Ca^{2+}-dependent proteolysis, ATP-ubiquitin-proteasome-dependent pathway and autophagic-lysosomal system. By contrast, data reported in the literature do not clearly show that protein synthesis is decreased in cachexia. As an example, in cancer patients either reduced or unchanged protein synthesis rates have been reported [2]. Of interest, the progression of cachexia from latent to refractory [3] is associated with an anabolic window that could be successfully exploited by nutritional interventions [4].

Reduced energy production and increased energy expenditure are frequently observed in patients with cachexia, that generally present with resting energy expenditure above the physiological level. Crucial in this regard are muscle mitochondria, that show both morphological and functional alterations, these latter being mainly characterized by mitochondrial uncoupling and

reduced oxidative capacity [5]. Impaired mitochondria lead to decreased production of ATP that translates into an energy deficit, eventually resulting in loss of muscle mass and strength.

The depletion of muscle mass occurring in chronic diseases is very frequently associated with systemic inflammation. In particular, the balance between pro- and anti-inflammatory cytokines is shifted in favor of the former. In this regard, increased TNFα and TNF soluble receptor in the blood have been reported in chronic heart failure or cancer patients [6][7][8]. On the other side, genetic ablation of IL-10 in mice leads to loss of muscle mass [9], and the activation of transcription factors such as NF-κB and STAT3, well known targets of pro-inflammatory mediators, has been shown to result in muscle atrophy[10][11], mainly in view of enhanced protein breakdown rates. However, also the anabolic side of muscle protein metabolism could be affected by inflammation. Indeed, TNFα has been shown to inhibit the signaling pathways dependent on insulin, IGF-1[3][12], myostatin [3][13] or bone morphogenetic proteins[14]. These latter in particular, belong to a sophisticated network of signals that regulate the activation of the transcription complex Smad1/5/8. The same study has also demonstrated that myostatin and BMP-dependent pathways are alternatively activated, since both compete for Smad4. Inhibition of BMP signaling is able to revert muscle hypertrophy that characterizes mice lacking myostatin, suggesting that these signaling cascades are physiologically balanced to maintain muscle homeostasis [14]. Consistently, activation of BMP signaling in mice bearing the C26 colon carcinoma results in protection against muscle wasting (unpublished data).

Impaired myogenesis has also been proposed to contribute to muscle wasting, both in cancer cachexia and in aging-associated sarcopenia. Indeed, accumulation of $Pax7^{+}$ myogenic precursors unable to form myotubes has been reported in the muscle of tumor-bearing mice [15][16], while a decline in muscle stem cell number and function occurs in sarcopenic muscles [17 and refs. therein].

2. Therapeutic strategies for cachexia: the state of the art

So far, several therapeutic options have been proposed to treat cachexia, mainly deriving from both experimental and clinical studies. In view of the multifactorial pathogenesis of the syndrome, very different approaches have been described, including nutritional support, anti-inflammatory drugs, orexigenic compounds, anabolic hormones, microbiota modifications and exercise.

The lack of adequate nutritional intake is a common finding in patients with heart failure, cancer, chronic obstructive pulmonary disease (COPD) and aging [1]. Along this line, nutritional interventions, better if personalized, will likely lead not just to improved patient nutritional status, but also to increased tolerance and response to treatments, finally resulting in good clinical outcome. This is particularly evident in cancer patients, where the adoption of a nutritional support regimen able to preserve patient body weight leads to increased tolerance to anti-cancer treatments, allowing to maintain the right dosage and timing [18]. However, despite the good results obtained with parenteral nutrition, there is clear-cut evidence that the enteral route should be preferred [1]. To this purpose, several studies have focused on agents able to stimulate appetite, such as glucocorticoids, amino acids, ghrelin analogues, or progestins (megestrol acetate) [19]. These latter have been shown to improve anorexia by still unknown mechanisms likely including induction of neuropeptide Y, a factor that regulates appetite at the central level, and down-regulation of pro-inflammatory cytokines. Along this line, progestins are widely used to treat cancer cachexia, alone or as component of multimodal approaches [20].

Another strategy to improve caloric intake relies on ghrelin. This is an orexigenic peptide that is also able to regulate energy metabolism and that has been proposed as an approach to treat cancer cachexia. In this regard, ghrelin administration to tumor-bearing animals results in improved food intake as well as body and muscle weight both in the presence and in the absence of chemotherapy [21]. These observations have led to design a number of clinical trial to test ghrelin effectiveness also in cancer patients. The most relevant ones are those investigating the effects of anamorelin, a ghrelin analogue. The results of these trials do not provide clear-cut evidence about

anamorelin effectiveness to treat cancer cachexia. Indeed, while positive results have been reported in non-small cell lung cancer patients, the ROMANA trials have shown that while anamorelin improves body weight loss and FAACT score in lung cancer patients, it is not able to rescue the loss of hand grip strength [22][23]. Subsequent investigations have shown that, in selected groups of patients, anamorelin also leads to improved performance status [24]. Other appetite stimulants such as macimorelin and a synthetic human ghrelin are actually under investigation [20].

Taking into consideration the relevance of inflammation to the pathogenesis of cachexia, it is quite conceivable that a large number of studies have focused on anti-inflammatory drugs. Most of the work has been performed on experimental models, however clinical studies do exist. A randomized trial aimed to test the TNFα inhibitor thalidomide in pancreatic cancer patients with cachexia has proved effective in improving the loss of body weight and lean body mass. These results, however, has not been confirmed by a subsequent trial, while lenalidomide, a thalidomide analogue is currently under phase II investigation. The poor effectiveness of anti-TNFα strategies in preventing/delaying cancer cachexia also results from trials using monoclonal antibodies directed against this cytokine (infliximab). Other anti-inflammatory strategies involve the use of antibodies against IL-6, that have been reported to improve anemia and muscle wasting in pancreatic cancer patients. Of interest, targeting both TNFα and IL-6 with OHR118 has been shown to increase body weight and performance status in advanced cancer patients [20]. On the whole, the results show that, at least in the former settings, non-steroidal anti-inflammatory drugs or molecules able to directly interfere with cytokine bioactivity (specific antibodies, pentoxifylline, thalidomide) appear to positively impact on cachexia, even if it is unlikely that they can be used alone. Rather, their inclusion into combined therapeutic approaches to cachexia appears more concievable.

Several strategies have been proposed so far as a mean to counteract hypercatabolism and/or hypoanabolism. Along this line, approaches aimed at stimulating insulin, IGF-1 or androgen-dependent signaling pathways, or based on supply of amino acids and their derivatives (glutamine, leucine, β-hydroxy-β-methylbutyrate), or involving β_2-adrenergic agonists (clenbuterol, formoterol)

or myostatin antagonists, have been tested with promising results, at least in experimental settings [19]. In this regard a phase II trial assessing the effects of a combination treatment regimen including formoterol and megestrol acetate in cancer patients with cachexia has shown improved muscle mass and function. These results, however, have been obtained studying a very small group of participants [20].

Non-steroidal selective androgen receptor modulators (SARMs) such as enobosarm have been proposed as a tool to improve anabolism. In phase II trials enobosarm has been reported to improve the loss of lean body mass, quality of life and performance status in patients affected by various types of tumors. Preliminary data from a phase III trial show increase in both lean body mass and muscle function [20].

Treatments able to improve myogenesis have been proposed to valuably contribute to combination protocols aimed at counteracting the catabolic drive that characterizes muscle atrophy in cancer and aging. Indeed, tumor-bearing mice administered a MEK inhibitor show a reduced accumulation of myogenic ($Pax7^{+}$) cells in the muscle, that is associated with improved muscle strength and mass [15][25]. Consistently, improved muscle trophism has been reported in cholangiocarcinoma patients treated with the MEK inhibitor selumetinib [26].

Another important line of intervention, mainly aimed to improve energy metabolism, is represented by strategies targeting mitochondria. In this regard, one of the most relevant is exercise. There are two types of training, namely resistance and endurance exercise, although pure protocols are almost nonexistent; the former enhances force production without impinging on mitochondrial function, while the latter induces metabolic adaptations by increasing the number of mitochondria and stimulating the shift from glycolytic to oxidative metabolism.

Despite many different approaches have been proposed to improve cachexia, this syndrome is still basically untreatable. One possible reason is that most of the interventions above impinge on a rather restricted spectrum, targeting specific pathways that do not necessarily cover the wide range of alterations that occur in cachexia. Just as an example, the pharmacological or genetic

inhibition of specific proteolytic systems does not improve cancer-induced muscle wasting [27][28]. Therefore, there is the possibility that approaches addressing cachexia from a more 'general' point of view could reveal useful. Particularly intriguing in this regard is the possibility to interfere with the epigenetic regulation of gene expression, that involves different pathways among which DNA methylation, nucleosome positioning and histone modifications. Since aberrant epigenetic regulation has been associated with several diseases, drugs able to act as epigenetic modifiers are actively searched. Among such drugs, histone deacteylase (HDAC) inhibitors are quite interesting, being able to improve both myogenesis and muscle phenotype in dystrophic mice [29].

3. HDACs in the skeletal muscle

Histone acetylation and deacetylation respectively result in increased or decreased cell transcriptional activity. Such balance mainly depends on the activity of two classes of enzymes, namely histone acetyltransferases (HATs) and HDACs [30]. In addition to histones, HDACs and HATs can also act on non-histone proteins. As an example, the cellular localization (activation/inactivation) of transcription factors such as Signal Transducer and Activator of Transcription 1 (STAT1), Nuclear Factor-κB (NF-κB), p53 and FOXO can be affected by acetylation of specific lysine residues. Not only, the acetylation state can modulate protein stability, for instance by inhibiting their ubiquitylation and subsequent degradation by the proteasome.

Three groups of HATs [31] and 18 HDACs, organized into four classes (I-IV), have been described so far (Table 1), based on structure, function and phylogeny. Class I, II and IV HDACs are considered as 'classical' Zn^{2+}-dependent HDACs, while class III HDACs (sirtuins; SIRT) use NAD+ as a cofactor. HDACs included into class I and class IV have a nuclear localization, class IIa HDACs mainly reside in the cytoplasm and class IIb HDACs can be found both in the nucleus and in the cytoplasm, shuttling between the two cell compartments. Class III HDACs can be found in both the nucleus and cytoplasm, but also in mitochondria [31][32].

Class I HDACs are ubiquitous and have been found in many transcriptional corepressor complexes; their activity is mainly exerted by inhibiting transcription factors such as Sp1, p53 and the Rb protein. Class IIa and class IIb HDACs contribute to signal transduction pathways; phosphorylation causes in the former the exposure of a nuclear export sequence that allows the interaction with 14-3-3 proteins and the export to the cytoplasm. Class IIb HDAC6 and HDAC10 have been reported to deacetylate tubulin and proteins involved in autophagy [28 and refs. therein]. SIRT have been shown to contribute to biological processes such as apoptosis, DNA repair and autophagy, but also to play a role in the regulation of lifespan, at least in lower eukaryotes [33]. Consistently, aging, but also several chronic diseases, have been associated with altered SIRT activity [34]. HDAC11 has been discovered in 2002 and is the unique member of class IV HDACs. Its intracellular localization may vary in relation with cell type and environmental cues [35].

HDACs, in particular HDACs 1, 4, 5, 6 and SIRT1, have been proposed to critically regulate the onset and progression of skeletal muscle atrophy. In this regard, HDAC2, HDAC4, HDAC6 and SIRT1 mRNA expression has been found increased in the skeletal muscle of animals exposed to nutrient deprivation, denervation or cast immobilization. The same study reports that only cast immobilization and denervation are associated with increased mRNA levels of HDAC1 and HDAC3. By contrast, decreased HDAC7 and HDAC9 levels have been observed [36]. These observations are in agreement with previous reports showing that HDAC4 and HDAC6 contribute to the regulation of skeletal muscle mass. Overexpression of the former, in particular, has been shown to result in reduced myofiber cross sectional area [37], while its ablation improves phenotype in denervated muscles [37][38].Similarly, HDAC6 is overexpressed during muscle atrophy and its inactivation has been shown to protect against denervation-induced muscle atrophy [39]. HDACs have also been proposed to play a role in the regulation of atrogene expression, such as the muscle-specific ubiquitin ligases MurF1 and atrogin1/MAFbx, acting on two transcription factors that are relevant to muscle atrophy, namely myogenin and FoxOs. Indeed, while acetylated FoxO3 is translocated from the nucleus to the cytoplasm and subsequently degraded, its

deacetylation results in increased transcriptional activity [40]. Another feed-forward mechanism also occurs, HDAC6 expression in atrophic muscles being regulated by FoxO3 [39]. Myogenin, one of the muscle regulatory factors overexpressed in late myogenesis, also participates to the induction of atrogin1/MAFbx during denervation, with a mechanism that requires HDAC4 [38]. Along the same line, reduced total HDAC activity and reduced HDAC2 and HDAC5 mRNA have been reported in macrophages of patients affected by COPD [41]. More recently, reduced HDAC2 expression has been observed also in the muscle of COPD patients; such reduction finely correlates with muscle weakness [42].

Among the SIRT family, SIRT1 is involved in the regulation of energy homeostasis, being able to stimulate cell survival, to enhance insulin production, and to improve carbohydrate metabolism, lipid homeostasis and mitochondrial mass and quality [34]. Consistently, caloric restriction induces SIRT1 expression[34] and SIRT1 activity is enhanced by AMPK, a kinase that works as a cell energy sensor [43]. SIRT1 genetic ablation in mice is associated with systemic inflammation and metabolic alterations[44]. By contrast, overexpression of SIRT1 reduces the metabolic impact of high fat diet [45], partially inhibits the catabolic drive induced in the skeletal muscle by fasting or denervation [46] and improves muscle phenotype in *mdx* mice[47]. Along the same line, SIRT activation in aged mice results in improved muscle metabolism, likely in view of the induction of the master regulator of mitochondrial biogenesis peroxisome proliferator-activated receptor gamma coactivator (PGC)1-α [31].

Finally, in several pathological states associated with muscle atrophy, HDACs have been shown to regulate and to be reciprocally regulated by microRNAs (miRs; [31]). As an example, decreased muscle-specific miR levels and increased class II HDACs have been shown in muscle biopsies of patients affected by congenital myopathies [48].

4. HDAC inhibitors

HDAC inhibitors regulate gene expression by modulating the binding to DNA of transcriptional activators/repressors. Several HDAC inhibitors, of both synthetic and natural origin, such as molecules isolated from bacteria (trichostatin A, FK322), vegetables or marine organisms, are actually available. On the basis of their chemical structure, these inhibitors can be classified into five different groups (Table 2; [49]). With the exception of sirtuin inhibitors, most of them interact with the HDAC Zn^{2+} domain through a zinc-binding group connected to a cap and target different HDAC classes (see above) or specific classes/isoforms. As an example, hydroxamic acid derivatives are rather non selective inhibitors, being active on both class I and class II HDACs, and displaying poor isoform specificity; nevertheless, they were the first type of HDAC inhibitors to obtain FDA approval. This class of inhibitors includes, among others, trichostatin A (TSA) and vorinostat, also known as SAHA (suberoylanilide hydroxamic acid). While the former has never been used in the clinical practice due to its toxicity and metabolic kinetic, SAHA was approved by FDA more than ten years ago as a treatment for T cell lymphoma. From SAHA entry among the accepted drugs, only other four molecules (belinostat, panobinostat, romidepsin and chidamide) received approval from regulatory agencies for the treatment of hematological tumors. Two of them (belinostat and panobinostats) belong to the same family of SAHA and TSA, while romidepsin, a cyclic peptide, and chidamide target class I HDACs. Along this line, several other HDAC inhibitors are currently under investigation in clinical trials for the treatment of various cancer types [49].

Moreover, several pharmacological agents, some of them currently used in the clinical practice, have been reported to inhibit HDACs. As an example, valproic acid (VPA), a short chain fatty acid used for longer than five decades as mood-stabilizers and anti-epileptic drug, was discovered to inhibit both class I and II HDACs, the former more selectively than the latter. Similarly, HDAC inhibitory activity was reported for the 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitor atorvastatin [50]. A detailed description of the different HDAC inhibitors actually available can be found in excellent recent reviews [49][50][51].

The research on HDAC inhibitors was initially developed to improve anti-cancer treatment, since low histone acetylation was frequently reported in cancer cells and interference with HDACs exerts anti-proliferative action and leads to cell death by apoptosis [52]. This latter effect is also dependent on the up-regulation of pro-apoptotic molecules such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Death Receptor 5 (*DR5*), while pro-survival factors are down-regulated [53]. Moreover, HDAC inhibitors enhance the immune response against tumor by up-regulating the expression of both HLA and tumor-specific antigens as well as of and co-stimulatory molecules [53][54]. While currently investigated mainly as anti-cancer drugs, HDAC inhibitors have been proposed as useful tools in a wide variety of pathologies, such as diabetes, heart failure, kidney disease and neurological disorders [50][51]. In this regard, several HDAC inhibitors were also studied in pre-clinical models of autoimmune diseases, showing reduced inflammation, mainly due to restoration of normal cytokine levels. HDAC inhibition was also reported to reactivate latent HIV, improving the effectiveness of antiviral therapies and resulting in depletion of HIV reservoir [55].

5. Are HDAC inhibitors useful to counteract muscle wasting in cachexia?

Several lines of evidence suggest that HDAC inhibitors could be a pharmacological tool suitable for preventing or at least delaying muscle wasting in chronic diseases. Such an effect could be obtained targeting specific mechanisms, some of which will be addressed here below.

Interference with myostatin signaling

Myostatin, a protein belonging to the TGFβ superfamily, is a negative regulator of muscle mass. Indeed, lack of myostatin and/or impaired myostatin-dependent signaling result in muscle hypertrophy, while myostatin overexpression/overactivation are a frequent finding in conditions associated with loss of muscle mass and function. Along this line, the inhibition of myostatin and/or ligands of the same family such as Activin A (see below), could be relevant as treatment options in diseases associated with muscle wasting [56].

Broad-spectrum HDAC inhibitors such as VPA, TSA, or sodium butyrate have been shown to down-regulate the signaling dependent on myostatin. In this regard, TSA has been shown to induce myostatin expression in C2C12 myotube cultures by modulating both ASK1-MKK3/6-p38 MAPK and ASK1-MKK4-JNK signaling pathways [57]. In addition, muscle follistatin levels have been increased by treatment with HDAC inhibitors, resulting in down-regulation of myostatin activity and improved myoblast recruitment. Along this line, the dystrophic phenotype occurring in *mdx* mice has been improved by treatment with TSA. Such improvement is associated with increased follistatin expression and, likely, with inhibition of GSK-3β [58]. Consistently, both myostatin and follistatin levels are normalized in the muscle of tumor-bearing mice; however, such effect is not paralleled by improvement of muscle wasting [59].

While TSA down-regulates myostatin levels, SAHA has been shown to induce the expression and release of Activin A, leading to R-SMAD phosphorylation, down-regulation of p21 and inhibition of proliferation in melanoma cell lines [60]. Activin A shares the signal transduction pathway with myostatin, and its levels are frequently increased in cachexia [61]. In this regard, Activin A induction by SAHA would not be good, however, at present nobody has investigated if SAHA administration in conditions characterized by already high Activin A levels exerts the same effect.

Myogenesis improvement

Treatment with TSA stimulates myoblast recruitment and fusion with existing myotubes, likely improving myogenesis [29] and improved myogenic differentiation *in vitro* has been recently reported using MS-275, a selective class I HDAC inhibitor, combined with a NO donor [62]. Consistently, *in vitro* HDAC inhibition by sodium butyrate appears to promote satellite cell fusion into existing myotubes as well as to stimulate myotube hypertrophy [29]. Damaged muscles exposed to TSA show increased expression of markers of myogenesis, supporting a beneficial effect of HDAC inhibitors on muscle regeneration [29]. More recently, tributyrin, a butyrate derivative, has been shown to positively impinge on muscle growth by stimulating satellite cell recruitment,

differentiation and fusion into myotubes in neonatal piglets, but not in adult animals [63]. In addition, HDAC inhibition in murine models of muscle dystrophy increases the ability of fibro-adipogenic precursors to stimulate muscle stem cells [reviewed in 24].

HDAC inhibitors have been proposed to target genes involved in myogenesis, however they can also impinge on non-histone proteins such as the transcription factors MEF2 and MyoD, further sustaining their acetylation occurring during myogenesis [29]. In addition, myoblasts exposed to HDAC inhibitors before differentiation show enhanced myogenin and Myf5 expression [29]. On the whole, these observations suggest that HDAC inhibitors act on myogenesis at different levels, among which modulation of gene expression, acetylation level of muscle regulatory factors and follistatin availability.

Effects on muscle mass and function

TSA administration protects against unloading-induced soleus muscle wasting, mainly by preventing the increase of MuRF-1 expression [64]. The same inhibitor has been shown to result in increased survival and myofiber cross sectional area in transgenic mice modeling spinal muscular atrophy [65] and to improve both muscle atrophy and the alteration of the neuromuscular junction occurring in SOD1-G93A mice, an experimental model of amyotrophic lateral sclerosis (ALS). However, such protection has not been confirmed in ALS patients treated with valproic acid or phenylbutyrate [66] . This inconsistency could be due to the fact that the HDAC inhibitors used in the clinical studies have a rather poor selectivity, being able to inhibit most of the HDACs. Indeed, it is likely that specific HDACs participate to the onset and progression of ALS, while others might be required to maintain the homeostasis, and their inhibition could be detrimental. In this regard, a recent study has explored the effects of a new selective class II HDAC inhibitor, namely MC1568, on SOD1-G93A mice. The results show that MC1568 administration leads to improved motor activity, mainly associated with increased muscle electrical potential and expression of myogenic genes [67].

Myofiber cross sectional area, muscle force and exercise capacity in mdx mice are markedly improved by HDAC inhibitors; such improvement is associated with reduced muscle degeneration and fibrosis [58][68]. Similar beneficial effects have been reported in an experimental model of limb girdle muscle dystrophy due to the lack of α-sarcoglycan [29].

Experimental data show that butyrate administration improves sarcopenia of aging resulting in increased myofiber dimensions and reduced lipodystrophy. In addition, muscle energy metabolism is also improved, likely due to increased mitochondrial biogenesis and reduced oxidative stress [69].

Modulating cachexia impinging on tumor growth

Many HDAC inhibitors, among which TSA, SAHA and the novel inhibitor chidamide, have been shown to inhibit cancer cell proliferation, alone or combined with chemotherapeutic drugs [52 and refs. therein]. However, both TSA and VPA have proved unable to either impair *in vivo* tumor growth or improve cancer-induced muscle wasting in experimental murine models [59][71]. VPA, however, results in increased follistatin expression associated with reduced myostatin levels and SMAD2/3 phosphorylation [59]. In a subsequent study [72], improvement of experimental cancer cachexia has been reported using doses of VPA higher than those previously tested in [59] and [71]. The beneficial effects on muscle wasting reported by Sun et al. [72] have been ascribed to inhibition of C/EBPβ-regulated atrogin1/MAFbx expression. In agreement with the data reported above, administration of the novel HDAC inhibitor AR-42 to mice bearing the C26 tumor or the Lewis lung carcinoma (LLC) improves muscle wasting in the absence of decreased tumor burden [73]. A similar improvement of muscle mass depletion has also been reported in a $KP^{fl/fl}C$ transgenic model of pancreatic cancer, however such protection appears associated with significant reduction of tumor mass [70].

Recently, different types of epigenetic modifiers have been shown to exert synergistic anticancer activity. Indeed, enhanced apoptosis has been reported in rhabdomyosarcoma or in urothelial carcinoma cells concomitantly treated with the pan-BET inhibitor JQ1 and with HDAC

inhibitors such as SAHA, quisinostat or romidepsin [74][75]. These observations raise the possibility that the same combination could also be useful to improve cachexia. In this regard, recent observations show that treatment of mice bearing the C26 tumor with the BET inhibitor JQ1 partially prevents body weight loss and muscle wasting, also improving animal survival time [76].

6. Conclusions

Despite many promising results, there are still many challenges that limit the possibility to use HDAC inhibitors to treat cachexia. In this regard, most of the available data are obtained on experimental models and are mainly referred to the use of broad action HDAC inhibitors, while the number of clinical trials is still very low. Another important aspect is that the knowledge of the causative mechanisms of muscle wasting in cachexia is still far from being complete, limiting the rationale for the therapeutic use of HDAC inhibitors. In this regard, further research should clarify if specific HDAC isoforms are involved in cachexia and the mechanisms by which HDAC inhibitors may prevent the loss of muscle mass. The development of class-specific and isoform-specific HDAC inhibitors will help to uncover the role of HDACs in muscle wasting of cachexia, potentially resulting in the generation of useful pharmacological tools.

7. Expert Opinion

In the last years, novel HDAC inhibitors, endowed with increased selectivity and/or additional activity have been designed. As an example, a recent study reports the development of HDAC inhibitors able to donate acetyl groups, resembling the acetyl-donating property of aspirin. The biological activity of this new class of molecules has been tested on the tumor cell line MDA-MB-231, showing an inhibition of cell proliferation [77]. However, mimicking the mechanism of action of aspirin, these agents could be used as double edged swords in order to address two specific alterations of cachexia: they could be able to impinge both locally, by modulating the myostatin signaling pathway in the muscle (see above) and systemically, by interfering with inflammation.

Another point relevant to the potential beneficial effect of HDAC inhibitors on muscle wasting of cachexia is the observation that such drugs might behave as exercise mimicking agents. Indeed, exercise has been proposed to activate anabolic pathways and to down-regulate the activity of pro-inflammatory cytokines, eventually improving the loss of muscle mass and strength [78]. In the last years, molecules able to partially mimic the effects of exercise, such as PPARδ or AMPK agonists, SIRT1 activators and the metabolic modulator trimetazidine [79] have been successfully investigated. In this regard, mice administered Scriptaid, a drug that does not inhibit HDAC activity, but that is able to disrupt the class IIa HDAC corepressor complex, show increased food intake associated with enhanced oxidative metabolism and insulin-stimulated glucose uptake in the skeletal muscle, closely resembling the metabolic effects exerted by the exercise mimicking agents reported above [80].

Last but not least, few lines of evidence indicate that tumor-derived extracellular vesicles are emerging as an important route of cell-to-cell communication. In this regard, few years ago miR21-containing vesicles released by C26 colon cancer cells have been shown to mediate myoblast death, likely contributing to the onset of cancer-induced muscle wasting [81]. More recently, cachexia has been proposed to be mediated by tumors through the release of extracellular vesicles containing Hsp70/Hsp90 [82]. Along this line, the HDAC6 inhibitor tubacin specifically stimulates the release of $CD133^{+}$ vesicles from CaCo2 colon cancer cells, increasing their susceptibility to anticancer treatments [83]. While there are no data available in the literature, the possibility that inhibiting HDACs could exert positive effect on muscle mass depletion that characterizes cancer cachexia also by modulating the amount and/or the content of tumor-derived extracellular vesicles cannot be discarded.

While the observations reported above suggest that HDAC inhibition could be a useful strategy to counteract muscle atrophy (Figure 1), a note of caution must be introduced, since a recent study reveals that long term inhibition of HDAC4 might be detrimental in conditions such as

aging or neuromuscular diseases, unless this is not coupled with other pharmacological interventions such as the adoption of antioxidant treatments [84].

The rationale of using HDAC inhibitors in cachexia is to maintain the physiological level of gene transcription, preventing the overexpression of molecules that contribute to hypercatabolism, hypoanabolism, inflammation and impaired myogenesis. Along this line, it is likely that in order to be effective, HDAC inhibitors should be adopted as an early intervention, generating a sort of protective shield that renders chromatin not permissive to enhanced transcriptional activity. Consistently with this hypothesis, the positive effects induced in SOD1-G93A mice by MC1568 are lost in late disease stages [67].

The results obtained testing HDAC inhibitors in experimental models of muscle wasting have led, so far, to only two registered clinical trials in the http://clinicaltrials.gov platform, one testing valproate and levocarnitine in children with spinal muscular atrophy (NCT01671384), having changes in muscle force as primary outcome, change in forced vital capacity and valproate side effects as secondary outcomes and the other administering sodium valproate for glycogen storage disease type V (NCT03112889), with changes in VO2 peak as primary outcome and presence of phosphorylase positive fibres, change in total walked distance (12 min walk), blood lactate and quality of life as secondary outcomes. Of these trials, the former appears still recruiting subjects, while the latter has been completed in February 2018 and not yet published. The limited translation of experimental results into clinical trials likely reflects the lack of solid evidences of HDAC inhibitor effectiveness in preventing/delaying the onset of cachexia.

Further investigation uncovering the pathogenetic mechanisms of muscle wasting coupled with the identification of suitable HDAC inhibitors targeting such alterations (Figure 2) will potentially lead to new trials. If the results obtained in both experimental and clinical settings will be confirmed, HDAC inhibitors may become components of a multimodal preventative treatment for cachexia, in particular that frequently occurring in cancer patients. The pathogenesis of cancer cachexia is multifactorial, and no effective therapeutic strategies are actually available. In this regard, a

selectively tailored multidirectional approach to cachexia is gaining a growing consensus and could benefit from the introduction of HDAC inhibitors.

Disclosure

The authors declare that there is no conflict of interest.

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** **very nice study suggesting potential limitations to the long term use of HDAC inhibitors**

Table 1. Classification of histone deacetylases (HDACs) in mammals

Family	**Class**	**Name**	**Localization**
Histone deacetylase	Class I	HDAC1	Nucleus
		HDAC2	Nucleus
		HDAC3	Nucleus
		HDAC8	Nucleus/cytoplasm
Histone deacetylase	Class IIa	HDAC4	Nucleus/cytoplasm
		HDAC5	Nucleus/cytoplasm
		HDAC7	Nucleus/cytoplasm
		HDAC9	Nucleus/cytoplasm
Histone deacetylase	Class IIb	HDAC6	Cytoplasm
		HDAC10	Cytoplasm
Sir2 regulator	Class III	SIRT1	Nucleus/cytoplasm
		SIRT2	Cytoplasm
		SIRT3	Mitochondria
		SIRT4	Mitochondria
		SIRT5	Mitochondria
		SIRT6	Nucleus
		SIRT7	Nucleus
Histone deacetylase	Class IV	HDAC11	Nucleus/cytoplasm

SIRT = Sirtuin

Table 2. Structural classification of histone deacetylase (HDAC) inhibitors

Class	**Examples**
sirtuin inhibitors	nicotinamide
short chain fatty acids	sodium butyrate, valproic acid
benzamides	tubastatin A, entinostat
hydroxamic acid derivatives	trichostatin A, vorinostat (SAHA)
cyclic peptides	romidepsin

SAHA = Suberoylanilide Hydroxamic Acid

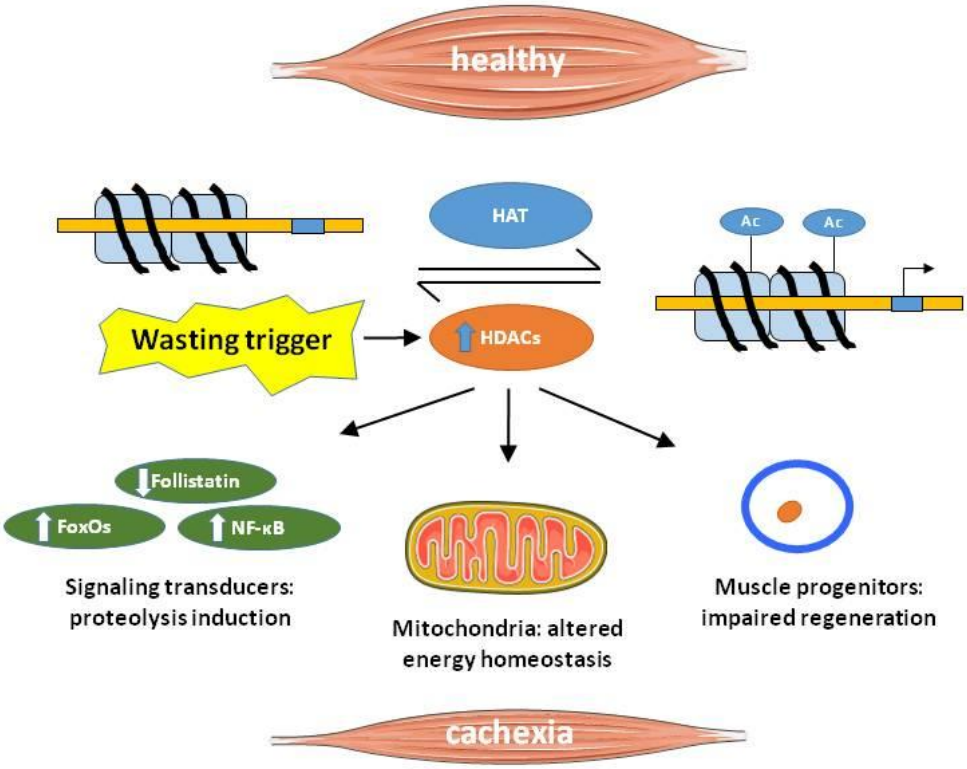

Figure 1. HDAC potential targets in muscle wasting. The balance between histone acetyl transferase (HAT) and histone deacetylase (HDAC) activities contributes to the physiological rate of gene transcription. Cachexia is associated with wasting stimuli that alter the HAT/HDAC equilibrium, leading to overexpression and/or activation of transcription factors typically deregulated in cachexia, to impaired mitochondrial homeostasis and to defective myogenesis. These alterations ultimately result in loss of muscle mass and function.

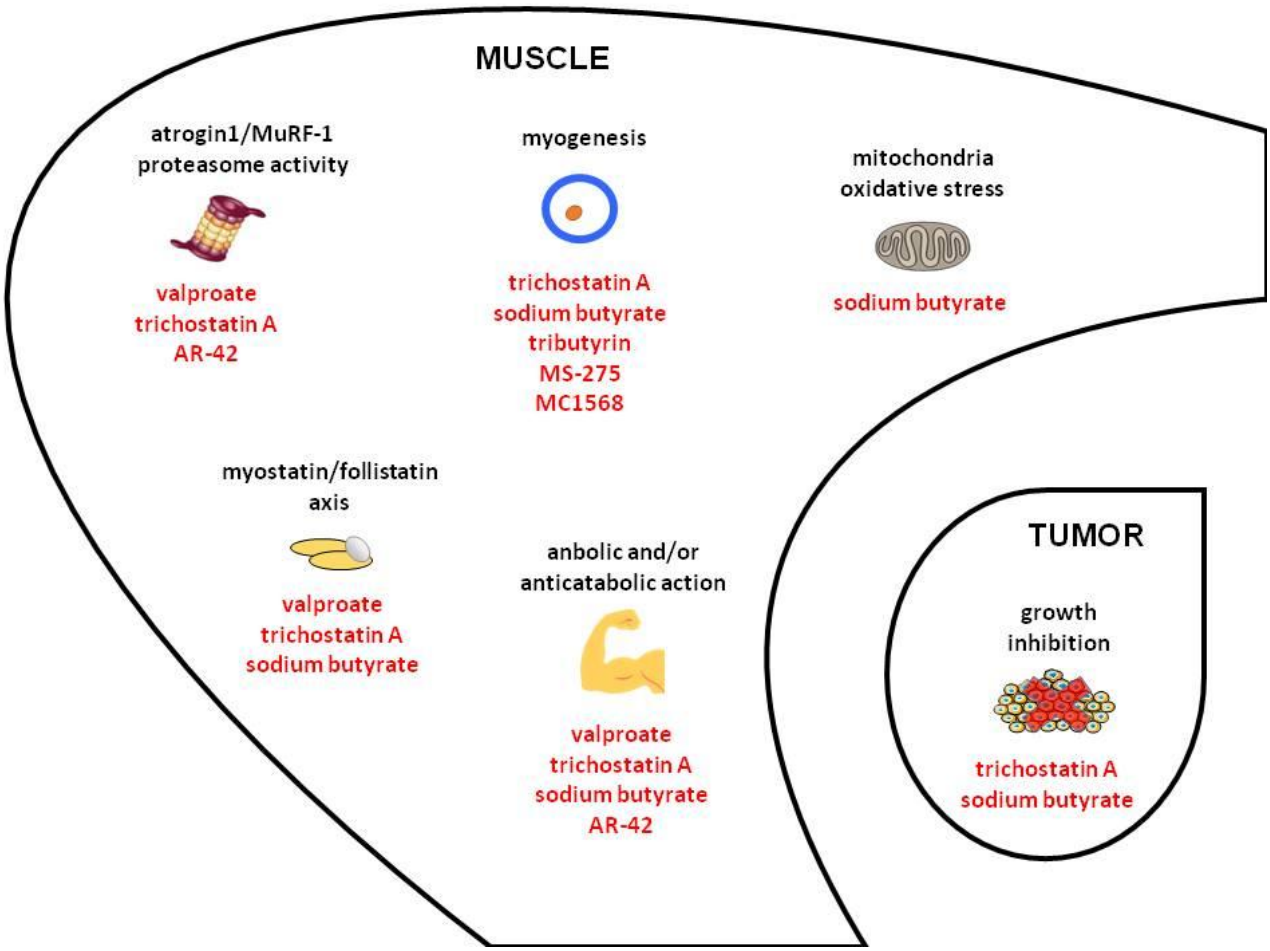

Figure 2. Molecular targets of specific HDAC inhibitors in cachexia. Distinct histone deacetylase (HDAC) inhibitors have been demonstrated to target several pathways/processes, potentially promoting muscle hypertrophy or counteracting muscle wasting. Given that specific HDAC inhibitors also exert anti-tumor activity, the simultaneous anabolic/anticatabolic action represents a desirable additional effect possibly allowing to treat both cancer and the development of cachexia with the same molecule.